Supplemental information

Differential arrival of leading and lagging strand DNA polymerases at fission yeast telomeres

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Supplemental materials and methods

Yeast strains and plasmids

For *mcm2-HA*, *rad11-FLAG*, *trt1-myc* and *trt1Δ::his3*$^+$, original strains have been described previously (Nakamura et al., 1997; Noguchi et al., 2004; Snaith and Forsburg, 1999; Webb and Zakian, 2008). PCR-based methods (Bähler et al., 1998; Krawchuk and Wahls, 1999) were used to create carboxy-terminally tagged *pot1-myc*, *stn1-myc*, *ten1-FLAG*, *poz1-FLAG*, *pol1-FLAG*, *pol2-FLAG* and *pol3-FLAG* (Primer used are listed in supplemental Table S2). An amino-terminally tagged *myc-rad26* strain was created in four steps. First, the *kanMX6* marker was integrated at the 3' un-translated region to generate *rad26*$^+$*::kanMX6* strain. Second, *hphMX6* marker (Sato et al., 2005) was integrated to replace *kanMX6* to generate *rad26*$^+$*::hphMX6* strain. Third, *rad26*$^+$*::hphMX6* strain was transformed to integrate loxP-*kanMX6*-loxP-*9myc* fragment, PCR amplified from pOM20 plasmid (Gauss et al., 2005), to the promoter region of r*ad26*$^+$. Finally, *kanMX6* was excised by transforming loxP-*kanMX6*-loxP-*9myc-rad26+::hphMX6* strain with a PW7 plasmid which encodes the P1 bacteriophage Cre recombinase (Werler et al., 2003) to generate *9myc-rad26*$^+$*::hphMX6* strain. Based on cell growth rate, HU sensitivity and stable telomere length maintenance, all tagged strains were deemed largely intact in their functional roles for DNA replication, DNA repair, DNA checkpoint, and telomere maintenance (supplemental Figure S1 and data not shown). For telomere specific factors that showed cell-cycle regulated changes in telomere binding by ChIP assays (Pot1, Stn1, Trt1 and Taz1), Western blotting experiments were also performed on the samples collected from synchronized cell cultures to ensure that these proteins are expressed throughout the cell cycle (supplemental Figure S2). Monoclonal anti-myc (9B11; Cell Signaling), monoclonal anti-HA (12CA5, Roche) and monoclonal anti-Cdc2 (Y1004, Abcam) were used in these experiments. Previous studies have found that Pol1 (α), Pol3 (δ), Mcm2/Cdc19, and Rad11 (RPA) proteins are expressed throughout the cell cycle (Forsburg et al., 1997; Kibe et al., 2007; Park et al., 1993). Based on microarray analysis of mRNA expression

levels during the cell cycle (Rustici et al., 2004), Rad26, Nbs1, and Pol2 (ε) proteins are also expected to present throughout the cell cycle.

Cell cycle synchronization

Fission yeast cells carrying the *cdc25-22* mutation were grown overnight in 600 ml YES media (Alfa et al., 1993) at 25 ºC. Exponentially growing cells were shifted to 36 ºC for 3 hours, cooled to 25 ºC, and further cultured in the absence or presence of 15 mM HU or in the absence or presence of 50 μM BrdU. Cells were then collected and processed every 20 min. Septation index was also monitored to ensure comparable synchronization of cultures among all cell cycle experiments.

BrdU incorporation analysis

Genomic DNA was prepared from fission yeast cells expressing *hENT1* and *TK* genes and incubated with 50 μM BrdU. 200 ng of DNA was denatured at 95°C for 5min, immunoprecipitated with 1 μg anti-BrdU antibody (B44; Becton-Dickinson) on Dynabeads Protein G (Invitrogen). After extensive washes with TBSE (10mM Tris-HCl pH7.5, 150mM NaCl, 0.1mM EDTA), TBSE + 1% Triton X-100, and TE (10mM Tris-HCl pH7.5, 1mM EDTA), bead-bound DNA was recovered using Chelex-100 resin (Bio-Rad). For determination of relative precipitation values, see ChIP analysis sub-section in the materials and methods section of the main text.

Co-immunoprecipitation assays

Cells were grown in YES, disrupted by cryogenic milling using Retsch MM301 (frequency 30 1/S, 3 min running time per cycle, total 5 cycles), and resuspended in lysis buffer (50 mM Tris pH8.0, 150 mM NaCl, 10% Glycerol, 5 mM EDTA, 0.5% NP40, 1 mM DTT, 1 mM Na3VO4, 1 mM PMSF, and Roche complete protease inhibitor cocktail). Lysates were incubated with either monoclonal anti-myc (9B11; Cell Signaling) or monoclonal anti-FLAG (M2-F1804; Sigma) antibodies, and protein-antibody complexes were purified with Dynabeads Protein G (Invitrogen). Purified samples were analyzed on SDS-PAGE and subsequent Western Blot analysis using either monoclonal anti-myc (9B11) or monoclonal anti-FLAG (M2-F1804) antibodies.

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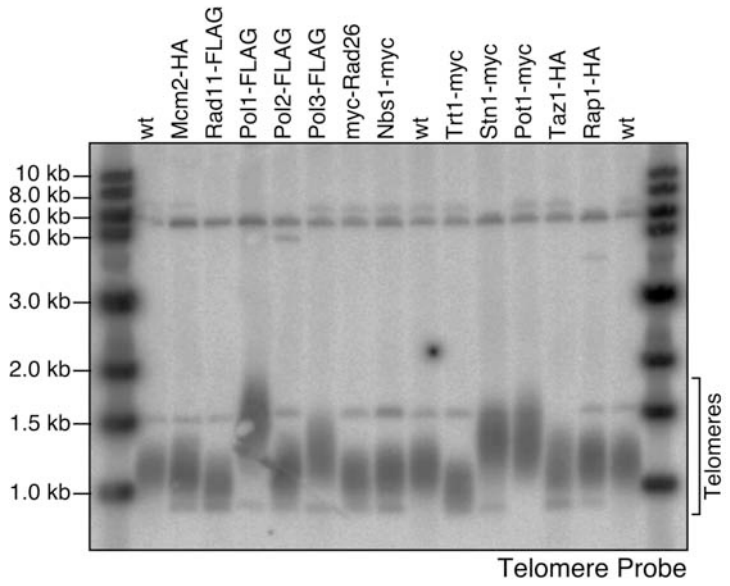
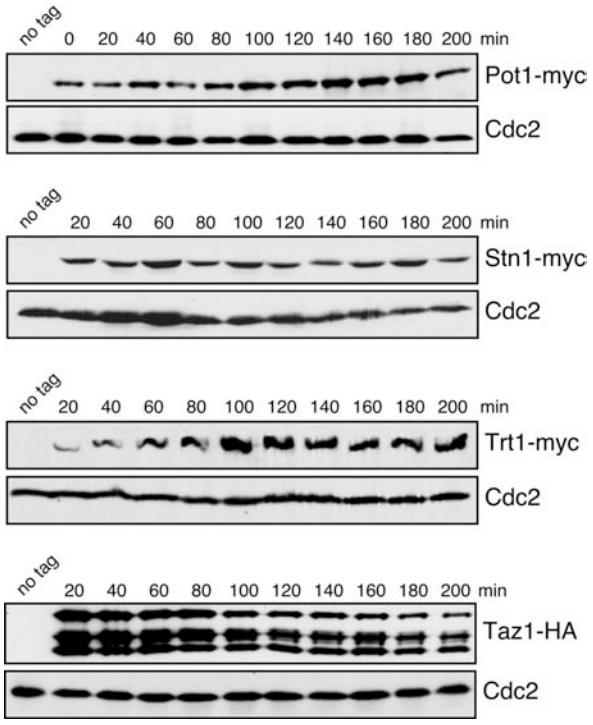
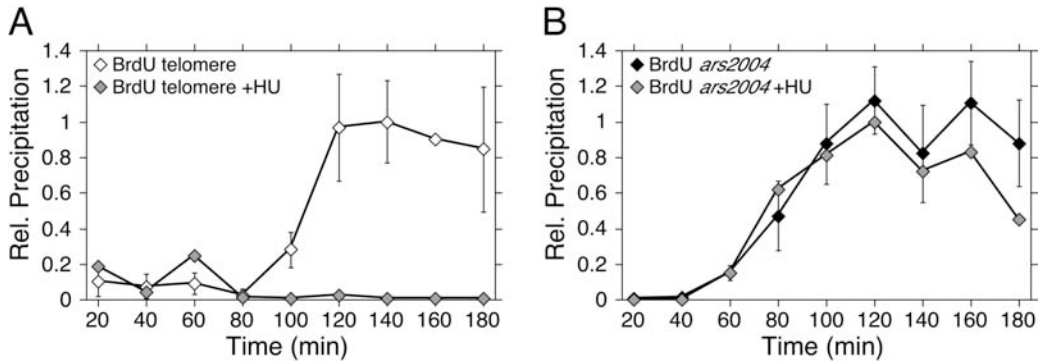


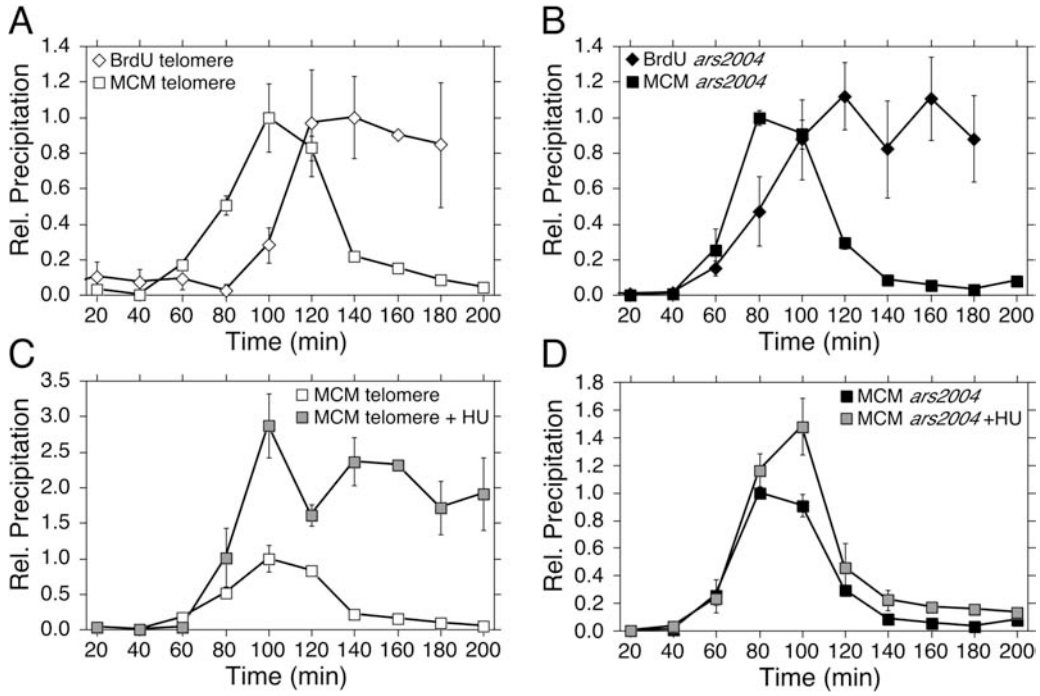
Supplemental Figure S1. Telomere lengths of indicated *cdc25-22* tagged strains used in this study. Strains were restreaked multiple times on YES plates incubated at 25 ^{o}C to ensure telomere length equilibrium prior to preparation of genomic DNA. After digestion with *EcoR*I, genomic DNA was subjected to electrophesis on a 1% agarose gel, transferred to a nylon membrane, and hybridized to a telomeric DNA probe (Nakamura et al., 1997).



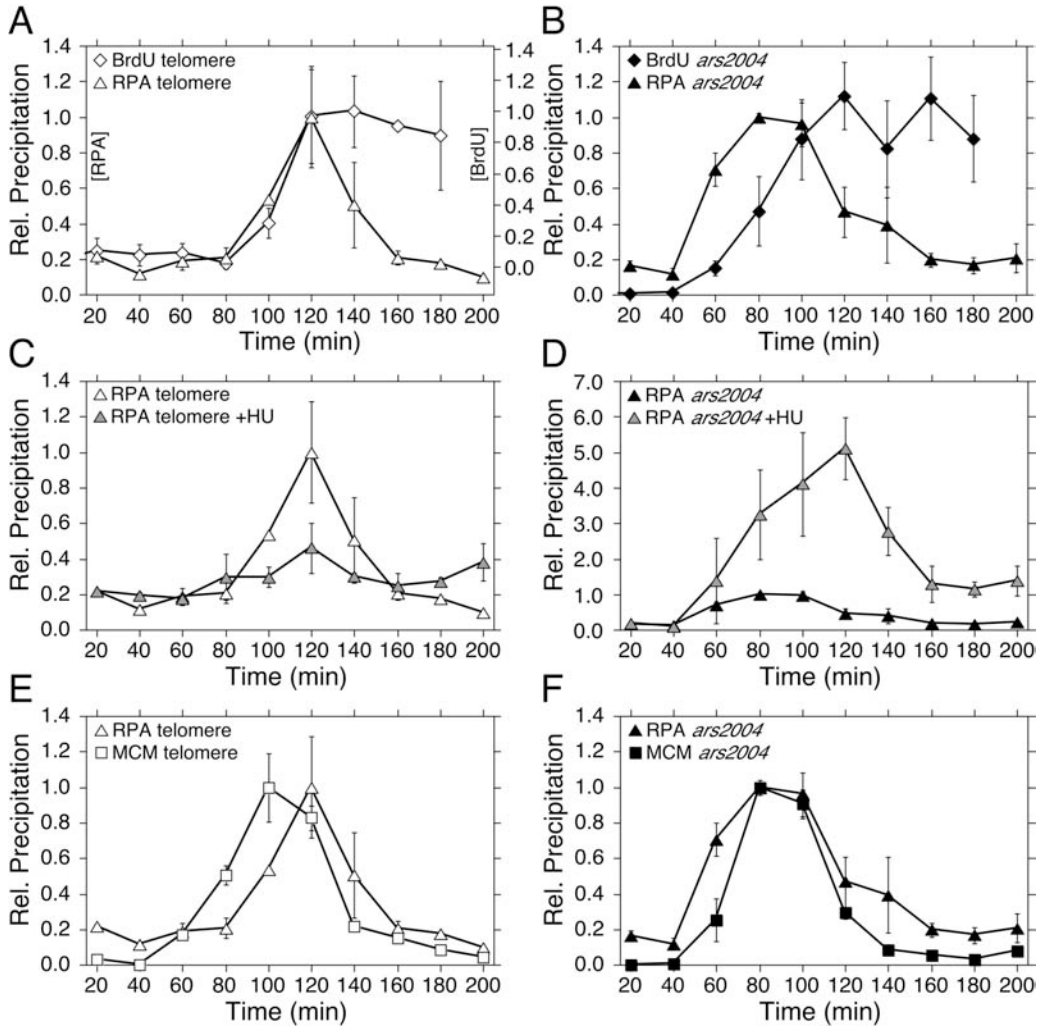
Supplemental Figure S2. Protein expression levels for indicated telomere specific proteins during *cdc25-22* synchronized cell-cycle experiments. After cells were synchronized in G_2/M at 36 ^{o}C for 3 h, cell cultures were shifted to 25 ^{o}C, cell pellets were collected at times after shift as indicated, and processed for Western blotting. Cdc2 was used as loading control.



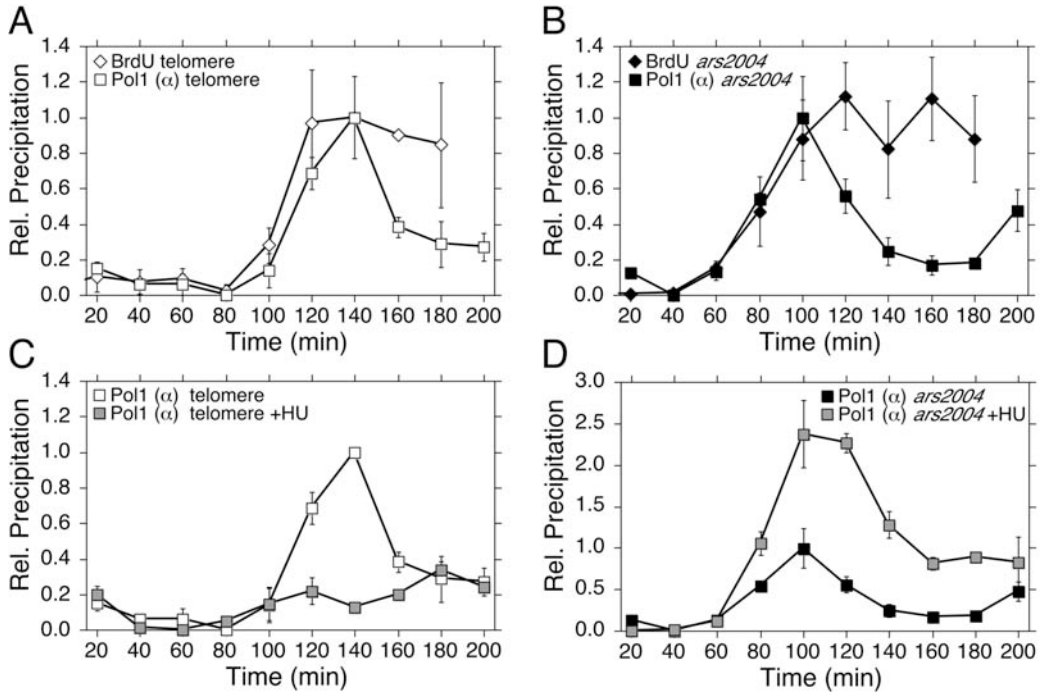
Supplemental Figure S3. DNA replication timing monitored by incorporation of BrdU in *cdc25-22* synchronized cells. **(A)** BrdU incorporation at telomeres is inhibited by addition of 15 mM HU. **(B)** BrdU is incorporated into *ars2004* with similar kinetics in the presence or absence of 15 mM HU. Error bars for non-HU data represent standard deviation from three independent experiments, while HU experiment was done once.



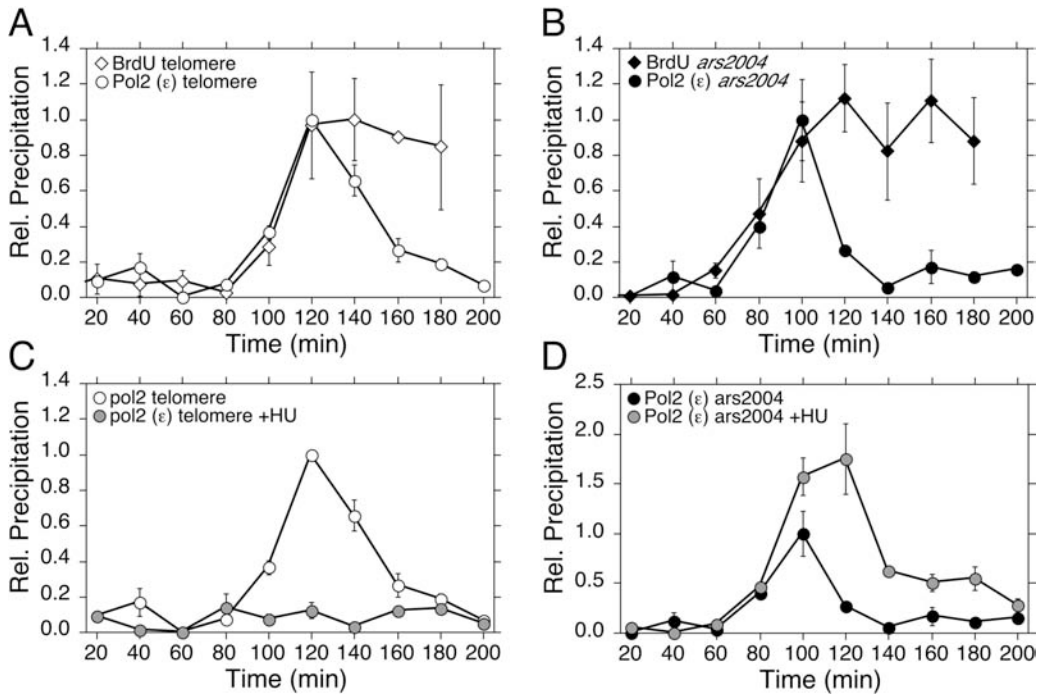
Supplemental Figure S4. Cell cycle regulated recruitment of MCM to telomeres and *ars2004.* For Explanation of error bars, see Figure 1. **(A, B)** Comparison of MCM recruitment (n=2) and BrdU incorporation (n=3) at telomeres (A) and *ars2004* (B). **(C, D)** Recruitment of MCM to telomeres (C) and *ars2004* (D) in the absence or presence of 15 mM HU (n=2).



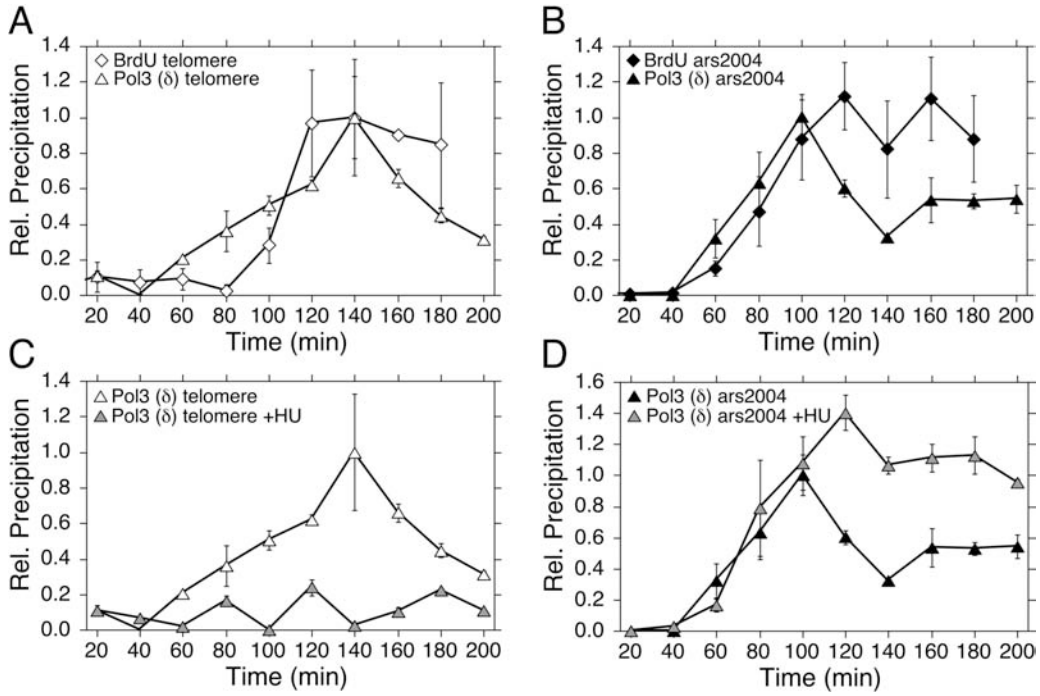
Supplemental Figure S5. Cell cycle regulated recruitment of RPA to telomeres and *ars2004.* For explanation of error bars, see Figure 1. **(A, B)** Comparison of RPA recruitment (n=2) and BrdU incorporation (n=3) at telomeres (A) and *ars2004* (B). **(C, D)** Recruitment of RPA to telomeres (C) and *ars2004* (D) in the absence or presence of 15 mM HU (n=2). **(E, F)** Comparison of recruitment timing to telomeres (E) and *ars2004* (F) for RPA and MCM. Data from Figure 1B (MCM) and 1C (RPA) are combined.



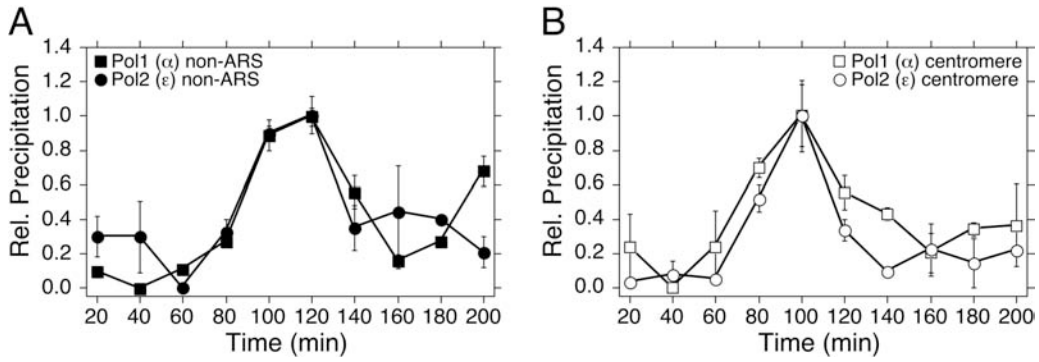
Supplemental Figure S6. Cell cycle regulated recruitment of Polα to telomeres and *ars2004.* For explanation of error bars, see Figure 1. **(A, B)** Comparison of Polα recruitment (n=2) and BrdU incorporation (n=3) at telomeres (A) and *ars2004* (B). **(C, D)** Recruitment of Polα to telomeres (C) and *ars2004* (D) in the absence or presence of 15 mM HU (n=2).



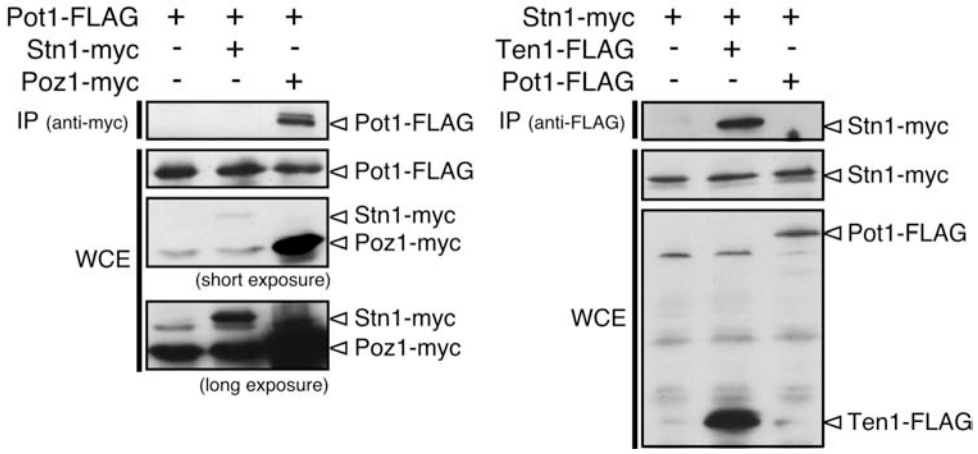
Supplemental Figure S7. Cell cycle regulated recruitment of Polε to telomeres and *ars2004.* For explanation of error bars, see Figure 1. **(A, B)** Comparison of Polε recruitment (n=2) and BrdU incorporation (n=3) at telomeres (A) and *ars2004* (B). **(C, D)** Recruitment of Polε to telomeres (C) and *ars2004* (D) in the absence or presence of 15 mM HU (n=2).



Supplemental Figure S8. Cell cycle regulated recruitment of Polδ to telomeres and *ars2004.* For explanation of error bars, see Figure 1. **(A, B)** Comparison of Polδ recruitment (n=2) and BrdU incorporation (n=3) at telomeres (A) and *ars2004* (B). **(C, D)** Recruitment of Polδ to telomeres (C) and *ars2004* (D) in the absence or presence of 15 mM HU (n=2).



Supplemental Figure S9. Polα and Polε are recruited with similar timing at Non-ARS region and centromeres. **(A)** Comparison of recruitment timing to non-ARS region (~30 kb Away from *ars2004*) for Polα (n=2) and Polε (n=2). **(B)** Comparison of recruitment timing to centromeres for Polα (n=2) and Polε (n=2).



Supplemental Figure S10. Pot1 and Stn1 do not form a stable complex. Pot1 can be co-immunoprecipitated with Poz1, a known Pot1 complex subunit of the Pot1 complex (left panel). Stn1 can be co-immunoprecipitated with Ten1, a known Stn1 complex subunit (right panel). However, we failed to detect any interaction between Pot1 and Stn1 by co-immunoprecipitation (both panels).

Supplemental Table S1: *S. pombe* strains used in this study

Tagged Protein	Strain	Genotype
no tag	TN1741	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 cdc25-22*
no tag (*hENT/TK*)	TN4777	*h⁻ leu1-32::[hENT1 leu1⁺] ura4-D18 his3-D1 his7-366::[hsv-TK his7⁺] cdc25-22*
Mcm2-HA	BAM7839	*h⁺ leu1-32 ura4-D18 ade6-M210 cdc25-22 cdc19Δ::[cdc19⁺-HA leu1⁺]*
Rad11-FLAG	BAM5875	*h⁺ leu1-32 ura4-D18 his3-D1 cdc25-22 rad11⁺-5FLAG::kanMX6*
myc-Rad26	TN7840	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 9myc-rad26⁺::hphMX6*
Nbs1-myc	TN7697	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 nbs1⁺::13myc-kanMX6*
Pol1-FLAG	TN4284	*h⁻ leu1-32 ura4-D18 cdc25-22 pol1⁺-5FLAG::kanMX6*
Pol2-FLAG	TN4782	*h⁺ leu1-32 ura4-D18 ade6-M210 his3-D1 cdc25-22 pol2⁺-5FLAG::kanMX6*
Pol3-FLAG	TN4434	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 pol3⁺-5FLAG::kanMX6*
Trt1-myc	TN7708	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 trt1⁺-G8-13myc::kanMX6*
Taz1-HA	BAM4643	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 taz1⁺-3HA::ura4⁺*
Rap1-HA	TN4688	*h⁻ leu1-32 ura4-D18 ade6-M210 his3-D1 cdc25-22 rap1⁺-3HA::LEU2*
Pot1-myc	BAM4650	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 pot1⁺-13myc::kanMX6*
Stn1-myc	TN6886	*h⁻ leu1-32 ura4-D18 his3-D1 cdc25-22 stn1⁺-13myc::kanMX6*
no tag	TN2411	*h⁻ leu1-32 ura4-D18 his3-D1*
Pot1-FLAG	BAM4295	*h⁻ leu1-32 ura4-D18 his3-D1 pot1⁺-5FLAG::kanMX6*
Stn1-myc	YTC6733	*h⁻ leu1-32 ura4-D18 his3-D1 stn1⁺-13myc::kanMX6*
Pot1-FLAG Stn1-myc	TN6944	*h⁻ leu1-32 ura4-D18 his3-D1 pot1⁺-5FLAG::kanMX6 stn1⁺-13myc::kanMX6*
Pot1-FLAG Poz1-myc	TN7011	*h⁻ leu1-32 ura4-D18 his3-D1 pot1⁺-5FLAG::kanMX6 poz1⁺-13myc::kanMX6*
Ten1-FLAG Stn1-myc	TN7503	*h⁻ leu1-32 ura4-D18 his3-D1 stn1⁺-13myc::kanMX6 ten1⁺-5FLAG-TEV-Avi::kanMX6*

Supplemental Table S2: DNA primers used in strain construction

Strain	Primer Name	Primer Sequence (5' to 3')
pot1-myc and *pot1-FLAG*	pot1-T7	CGCATTCAGCATCACATATCG
	pot1-B8(x)	GGGGATCCGTCGACCTGCAGCGTACGAAACAATTTTCGTGCCAAATCC[1]
	pot1-T9(y)	GTTTAAACGAGCTCGAATTCATCGATGATACAAAACTTACAATAATG[1]
	pot1-B10	GATATTTCACGTTTCCCCTC
stn1-myc	stn1-tagT	TTTGTCAATTTTTGCTTCGTACAAAAGGGAAATGGAGGCAAGCAAAGAAATATACCTGG GTGCGAGATAATCAGTTTGTACGGATCCCCGGGTTAATTAA[1]
	stn1-tagB	ATTAACCGCTTATATACCCATGTGTACTTATTGATCTGTTTCCGTAAACATATTCTTAA ATTAATAGAGGATTGTAATATGAATTCGAGCTCGTTTAAAC[1]
ten1-FLAG	ten1-T1	AGGATGCGTGCAATCATATAAGAATGGCAT
	ten1-B2(x)	GGGGATCCGTCGACCTGCAGCGTACGAATCACATTTTTGACGTTCAGAAACCATTT[1]
	ten1-T3(y)	GTTTAAACGAGCTCGAATTCATCGATAATCGTGTTAATGTCAGTCTTTATAAT[1]
	ten1-B4	TGTTGGAAAGACAAAACAAATTCTGGTA
poz1-myc	poz1-T3	AGACTGGGGAAGTCATAACGAA
	poz1-tag(x)	GGGGATCCGTCGACCTGCAGCGTACGAATTAATGTTTGAGGTAAGCATTTTAACA[1]
	poz1-tag(y)	GTTTAAACGAGCTCGAATTCATCGATTTTTGGTATCTTTAAATTCCTGGAG[1]
	poz1-B2	GCTCGTGCAATCGTAACAAATA
pol1-FLAG	pol1CT-5'	GTATGTGATGATTCTTCTTGTGG
	pol1CT-3'module	GGGGATCCGTCGACCTGCAGCGTACGACGATGAAAATATCAGTCCCATATC[1]
	Tpol1-5'module	GTTTAAACGAGCTCGAATTCTGAAGCTTTAATTCATTCTACCCGTTTAAG[1]
	Tpol1-3'	GTGAGTACTTTGCTATCCAGAGC
pol2-FLAG	pol2CT-5'	GTGTTTGAGGAAACTTTGGTGGATAAC
	pol2CT-3'module	GGGGATCCGTCGACCTGCAGCGTACGAGTTCAGCACAGAAAGTATGGACTG[1]
	Tpol2-5'module	GTTTAAACGAGCTCGAATTCTAACATTTCTCGCCTGACCATGAG[1]
	Tpol2-3'	CGAACGTTTAAGAGCATGAGTGG
pol3-FLAG	pol3CT-5'	TGAGACCTGTCTTGGATGCAAAGC
	pol3CT-3'module	GGGGATCCGTCGACCTGCAGCGTACGACCAGGACATTTCATCAAATCTTTTC[1]
	Tpol3-5'module	GTTTAAACGAGCTCGAATTCGATGGATGTTTTTAATTACTAAATGTG[1]
	Tpol3-3'	GCTGGCAAGTGTGCTTTGTCAGT
rad26^{+}::hphMX	rad26-delC-T	CTCCTTTCGTCAATGTACCCTCAAAATGAA
	rad26-wt-B	CTTATTTAGAAGTGGCGCGCCTCACTAAAAATTAGTGTACAACTGCTCC[2]
	rad26-T5	GTTTAAACGAGCTCGAATTCATCGATTACTTGTTTCTTCAGTGTTGTTCT[1]
	rad26-B6	TAGTGGTACTTTAATATATTCATTTTCGTT
	BAM96	GCTTGCCTCGTCCCCGCCGGGTC[1]
	kan-B5	GGCGGCGTTAGTATCGAATCGAC[1]
myc-rad26	rad26-T10	TAACTGTTAAGGTTTTCCAATTGC
	rad26-B11	TTTGTTTGTAGCTTTCACTCTCGT
	rad26-CreLox-T	AACAACTATTGTTACGCATAAACGAGAGTGAAAGCTACAAACAAAATGTGCAGGTCGAC AACCCTTAAT[3]
	rad26-CreLox-B	TTCATCCGAACCCAAACTCTCCAAGTCAAAACTTTCATCAGCCATGCGGCCGCATAGGC CACT[3]
	rad26-T12	ATGGCTGATGAAAGTTTTGACTTG
	rad26-B13	GATTCCCGGACTTTTTGAGAAGAA

[1]*kanMX6* sequence underlined. [2]*adh1* terminator sequence underlined. [3]pOM20 tagging module sequence underlined.

Supplemental Table S3: DNA primers used in ChIP assays

Location	Primer Name	Primer Sequence (5' to 3')	References
telomere	jk380	TATTTCTTTATTCAACTTACCGCACTTC	(Kanoh et al., 2005)
(TAS1)	jk381	CAGTAGTGCAGTGTATTATGATAATTAAAATGG	(Kanoh et al., 2005)
ars2004	ars2004-66-F	CGGATCCGTAATCCCAACAA	(Hayashi et al., 2007)
	ars2004-66-R	TTTGCTTACATTTTCGGGAACTTA	(Hayashi et al., 2007)
non-ARS	non-ARS-70-F	TACGCGACGAACCTTGCATAT	(Hayashi et al., 2007)
(~30 kb from *ars2004*)	non-ARS-70-R	TTATCAGACCATGGAGCCCATT	(Hayashi et al., 2007)
ars3002	ars3002F	TTGGCGCTAAACAATCTCTG	This study
	ars3002R	TCCTTGTCGAACTCAATTGC	This study
centromere	cen1-dh-1	CGAGCGATTTGAACATATGCATT	(Kanoh et al., 2005)
(cenH)	cen1-dh-2	AACGTACTGACCGATTTGATCGT	(Kanoh et al., 2005)